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Reprinted from JOURNAL OF DAIRY SCIENCE, August, 1964, Vol. XLVII, No. 8, page 922

OCCURRENCE OF EXTRACELLULAR ACIDIC ENDOPEPTIDASE OF AN AEROBIC SPORE-FORMING BACTERIUM

Endopeptidases are produced by many aerobic spore-forming bacteria, such as *Bacillus licheniforms* (1), *Bacillus mesentericus* (2), and *Bacillus subtilis* (3-6, 8). The endopeptidases obtained from most of these sources have been purified and characterized.

Among the various physico-chemical properties of these endopeptidases, their pH optimum for activity is of special interest. The pH optima of the enzymes from these sources are neutral or alkaline to about pH 10. The endopeptidases of such bacteria having pH optimum on the acidic side have not been reported. This preliminary report deals with the preparation and some properties of an acidic endopeptidase of bacteria designated as FR-2 (9), a variant of *B. subtilis*. The selection of this organism has added significance as Cheddar cheese prepared from the enzyme obtained from it was reported to be good and showed no bitter taste (9).

Bacillus subtilis was grown on an milk agar slant for 24 hr at 37 C. A saline suspension from 24-hr-old culture was used for inoculation. The organism was cultivated on a rotary shaker for 72 hr at 30 C. The fermented broth was filtered through Whatman No. 4 filter paper. The clear yellow liquid was precipitated by saturating with ammonium sulphate (BDH, Reagent Grade). The precipitated enzyme was dried in a vacuum desiccator. Yield was about 4 g per liter. A 10% solution of this dry enzyme in water was subjected to fractionation with ammonium sulphate. The proteolytic activity was determined according to the method of Kunitz (7), using Hammersten casein as the substrate. The protein content was obtained according to the method of Warburg and Christian (10).

Fraction obtained between 0.3 to 0.5 saturation had maximum specific activity (units per milligram protein). It was dialyzed 5 hr at about 8 C against phosphate buffer, 0.02 M, pH 5.5. The dialyzed liquor was then lyophilized. This preparation represented a purification of about 25-fold.

The pH optimum for activity of the enzyme (casein digestion) thus isolated was found to be 5.5. The enzyme was inactivated completely at 50 C in 15 min. It was not inhibited by versene (ethylene diaminetetraacetate, sodium salt) and sodium azide in concentration of 10^{-3} M. Potassium cyanide and p-chloromercuribenzoate (sodium salt) inhibited it completely in the same concentration. Lima bean and ovomucoid trypsin inhibitors (obtained from Sigma Chemicals, USA) inhibited the enzyme completely in 30 min in concentration of 20 μg at 30 C.

ACKNOWLEDGMENTS

This work is part of a project financed out of PL 480 funds. Grateful thanks are expressed to United States Department of Agriculture, Agricultural Research Service, Far Eastern Regional Research Office, American Embassy, New Delhi, India, and to the Government of India in the Ministry of Food & Agriculture. Thanks are also due to Dr. K. K. Iya, Director of Dairy Research Institute, Karnal, for his keen interest in the work.

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